REMARKS

Claims 1, 5-8, 10, 11, 13-15, 25, 26 and 28 are currently pending. Claims 1 and 13 are amended as discussed below. No new matter has been added herewith. The following addresses the substance of the Office Action.

Objection

Claim 13 was objected to because the claim recited "collecting the TALL-104 cells into frozen bags". The Examiner indicated that it would be more accurate for the claim to recite "collecting the cells into bags and freezing the bags. Applicants have amended the claim in accordance with the Examiner's suggestion to recite step (c) collecting the TALL-104 cells in to bags; and a further step (d) freezing the bags. Support for the amendment is found at page 9, line 10 of the Specification as filed.

Indefiniteness

Claim 6 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. In particular, the Examiner found that there was insufficient antecedent basis for the limitation "the harvest time" at line 2. Applicants have amended Claim 1, step (c) by substituting the term "recovering" with the term --harvesting--. In addition, Claim 6 is amended to recite "the harvesting step." Accordingly, the amended claims are in compliance with the requirements of 35 U.S.C. § 112, second paragraph.

Obviousness

The Patent Office has maintained the rejection of Claims 1, 5-8, 10-11, 13, 14, 25-26 and 28 under 35 U.S.C. § 103(a) as being unpatentable over WO 94/26284 in view of Gambacorti-Passerini et al. (1998 *Tumori* **74**:523-530), Tuyaerts et al. (2002 *J Immunological Methods* **264**:135-151) and Schumpp, B. and Schlaeger, E.J. (1990 *J Cell Science* **97**:639-647), as evidenced by the product information of Nunc cell factories. WO 94/262284 concerns the same T-lymphocyte cell line (TALL-104) of the present invention cells, but it refers to a method for modifying TALL-104 cells "to confer one or more desired characteristics that will make the cells suitable for use in marrow purging and in adoptive transfer immunotherapy in allogeneic (HLA-mismatched) recipients" (see, page 6, second paragraph of WO 94/262284). The Applicants respond to the rejection under 35 U.S.C. § 103(a) by addressing the following issues.

Antibiotic-Free Media

Referring to page 26, paragraph 1 of WO 94/26284, the Examiner stated that the reference teaches that the maintenance of TALL-104 cells is performed by culture in medium supplemented with 10% fetal bovine serum (i.e., a medium without antibiotics)." However, in the cited paragraph, there is no mention of an "antibiotic-free medium" or addition of only fetal serum. Instead, there is only an indication of what was notably added. In this regard, the Applicants point out that neither glutamine nor nonessential amino acids were specifically mentioned as well, although they are typically supplemented in any cell culture medium.

Contrary to the Examiner's assertion, there is no suggestion in WO 94/26284 to omit antibiotic. Instead, the emphasis in the disclosure of WO 94/26284 relates to a method for modifying TALL-104 cells. In the summary of the invention, from the first line of page 6 to the last line of page 7, all the disclosed aspects of the invention are reported, and there is no reference or citation to a method for amplifying TALL-104 cells in an antibiotic-free medium. Accordingly, the Applicants question how the skilled artisan would have been led to omit antibiotic from the culture, especially in view of the disclosure that same cells are originated by a medium with added antibiotics (as acknowledged by the Examiner on page 7, fourth paragraph). Moreover, WO 94/26284 cites and incorporates by reference Cesano and Santoli (1992 *In vitro Cell Dev Biol* **28A**:648, reference cited in IDS dated January 3, 2006) at page 19, lines 27-29 on page 26, paragraph 1. The Applicants wish to point out that Cesano and Santoli teach the use of the antibiotics on page 649 under the paragraph "Materials and Methods." Cesano and Santoli report that the antibiotic is always present in the medium as stated on page 649, under the paragraph "Materials and Methods" "*Cell cultures: TALL 103/2 and TALL-104 cells are routinely maintained at 37°C in 7% $C0_2$ in Iscove's modified Dulbecco's medium (IMDM) (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum, glutamine and antibiotics and 5 to 10 U/ml recombinant human (rh)IL-2 (Amgen, Thousand Oaks, CA)*" Therefore, in deciding to use TALL-104 cells the skilled person, in view of W094/26284, would have supplemented the medium with an antibiotic, which is specifically avoided by the present Claim 1 in order to amplify the cells.

Applicants respectfully suggest that the Examiner has misinterpreted the disclosure of W094/26284 in order to find all of the elements of the present Claim 1. However, this was only

possible because the Examiner knew the essential features of Claim 1. As such, the Examiner has made an improper hindsight reconstruction of the prior art based on the presently claimed invention.

As reported in MPEP 2142, "To reach a proper determination under 35 U.S.C. § 103, the Examiner must step backward in time and into the shoes worn by the hypothetical "person of ordinary skill in the art" when the invention was unknown and just before it was made. Moreover, MPEP 2142 states that impermissible hindsight must be avoided and the legal conclusion must be reached on the basis of the facts gleaned from the prior art." In the present case, the feature of Claim 1 of amplifying in an antibiotic-free medium can not be gleaned from W0 94/26284.

TALL-104 Cells Differ from Lymphocyte Activated Killer (LAK) cells and Natural Killer (NK) Cells

Gambacorti-Passerini et al. teach a method for the large scale production of Lymphocyte Activated Killer (LAK) Cells. Referring to page 13 of WO 94/26284, the Examiner indicated at page 7, last paragraph of the Office Action that TALL-104 cells simply represent a clonal population of cytotoxic cells that are also found within the heterogeneous LAK cell population" by. Hence, the Examiner concluded that the skilled artisan would have been motivated to apply the teachings of Gambacorti-Passerini to WO 94/26284. However, WO 94/262284 discloses that TALL-104 cells are a highly cytotoxic T-cell line established from a child with Acute Lymphoblastic Leukemia, which is an alternative to both Natural Killer (NK) cells and Lymphokine Activated Killer (LAK) cells for non-MHC mediated cell based cancer therapy. Therefore, it is well known that TALL-104, LAK and NK cells are functionally and morphologically different lymphocytes populations.

There are numerous differences between LAK, NK and TALL-104 cells as indicated in WO 94/26284. For example, several comparative data between TALL-104, LAK and NK are presented in Table II, page 15, and in Table III, pages 16-17 of WO 94/26284. In particular, among differences between LAK and TALL-104 cells, the Applicants note from page 13, line 37 to page 14, line 26, the following: "...after modification.... (e.g., after lethal irradiation (4000 rads) using a Cesium source) the TALL-104 cells, but not LAK cells, remain highly cytotoxic." Moreover, WO 94/26284 discloses at page 13, at Table I and in the first full

paragraph that "the lack of surface expression of CD16, a classical surface marker of NK cells, and the presence of a T cell receptor (TCR) with the α/β heterodimer, both indicate that these cells belong to the T cell lineage and not to the NK cell lineage." By contrast, WO 94/26284 reported that the antigenic profile of IL-2-expanded LAK cells include activated NK and T cells.

Referring to WO 94/26284 at page 14, lines 4-28, the TALL-104 cell line has a characteristic chromosomal translocation involving chromosomes 11 and 14, i.e., at t(11;14) (p13:q11). TALL-104 cell line is further characterized by TCR rearrangements alpha, beta, gamma and delta, and the ability to produce lymphokines such as GM-CSF, IFN-gamma, TNF-α, and TGF-β1. Moreover, the TALL-104 cell line is extremely cytotoxic against a broad spectrum of tumor cells and of virus infected cells. Specifically, the cell line displays potent MHC nonrestricted cytotoxicity against a broad variety of tumor cells (including human leukemias and solid tumors) (Tables II and III) and virus-infected cells (Table IV and Fig. 1), without damaging or killing cells from normal tissues. Tables II and III also show that conventional NK andLAK cells display a lower cytotoxic efficiency and a more restricted spectrum of activity against tumor and virus-infected targets, as compared to the TALL-104 cell line. Referring to Table II, WO 94/26284 reports that, importantly, the TALL-104 cell line is completely resistant to lysis by allogeneic NK and LAK cells and CTL, and thus would not be destroyed by the immune system of HLA-mismatched recipients."

Furthermore, the known heterogeneity of the LAK population, which is mainly composed of NK (i.e., Natural Killer cells), renders the teachings of Gambacorti-Passerini even less applicable to the clonal cell population of TALL-104, which are clearly not NK cells. Indeed, Gambacorti-Passerini confirms the heterogeneity of LAK cells on page 527, 2nd col. in the paragraph entitled "Activation of PBL in the presence of granulocyte contamination." TALL-104 cells are not NK cells, even if TALL-104 are simplistically considered as a clonal population of the cytotoxic T cells of the LAK population.

In summary, WO 94/26284 discloses a number of important differences between LAK or NK and TALL-104 cells. In addition to the disclosed differences from LAK cells and TALL-104 cells in WO 94/26284, there are even several significant differences between clonal isolates of the same original T-lymphocytic cell patient population, as shown by O'Connor et al., 1991 "Growth Factor Requirements of Childhood Acute T-Lymphoblastic Leukemia: Correlation

between Presence of Chromosomal abnormalities and Ability to Grow Permanently *in Vitro*" *Blood* 77:1534-1545, reference cited in IDS dated January 3, 2006). In particular, the attention of the Examiner is drawn to pages 1537-1539, wherein differences at phenotypic, genetic, immunologic and functional levels among TALL-101 through TALL-106 subclonal cell populations are reported. In view of these differences, the skilled artisan would not have applied the teachings of Gambacorti-Passerini et al. to TALL-104 cells.

The Examiner also stated at page 7 of the Office Action, last paragraph, lines 29-30 that "both LAK cells and TALL-104 cells are grown in a nearly identical manner by suspension culture with IL-2". However, the Applicants wish to point out that, although TALL-104 cells were reported to grow in suspension when in multiple flasks and in a lab scale, as described in several reports by the same authors of WO 94/26284, they could not be amplified by usual amplification means for "suspension" cells. This was shown in the present Specification as filed at pages 14-16, Example 2, where the growth in Spinner flasks and in a miniPerm fermentor, which are usually optimal for suspension cultures such as hybridoma cells exhibiting growth characteristics similar to TALL cells, was highly impaired, or even arrested.

Conversely NK cells, which primarily make up LAK cells, are reported to amplify in large scale in spinner-flask and in Bioreactors (i.e., as also demonstrated by Bryce A. Pierson et al. 1996 "Production of Human Natural Killer Cells for Adoptive Immunotherapy Using a Computer-Controlled Stirred-Tank Bioreactor" *Journal of Hematotherapy* **5**:475-483; this reference will be submitted subsequently along with an Information Disclosure Statement). Pierson et al. state in the abstract, lines 5-6: "We demonstrate that NK cultured in 250-ml spinner flasks expand 2.5-fold more than NK cultured in stationary tissue culture wells." At lines 10-11 of the abstract, Pierson et al. reports: "NK expansion in the 750-ml bioreactor was 7-fold greater than in stationary tissue culture controls and 3-fold greater than in spinner-flask controls." Therefore, Pierson et al. conclude that NK amplification in suspension, under agitation, is greater than in stationery tissue culture controls, such as in WO 94/26284.

In view of the teaching of WO 94/26284 and the relevant prior art at the time of filing, which reported evident differences not only between LAK cells, NK cells and TALL-104 cells, but also between the different subclones of TALL cells, the Examiner's has incorrectly concluded that a skilled person would have considered Gambacorti-Passerini et al. document,

which refers solely to LAK cells. To the contrary, given the unique characteristics of TALL-104 cells, which have been widely demonstrated at different levels by a number of reports, the skilled artisan would not have taken the disclosure of Gambacorti Passerini et al. into consideration because the reference describes a quite different LAK population.

In view of the foregoing, the skilled person, at the time of filing, would have motivated to try suspension cultures means (such as MiniPerm and Spinner flask), but not the Cell factory for TALL-104 amplification. In this regard, the skilled artisan would have failed since, as described in Example 2 of the present Application, TALL cells can not be grown under traditional large-scale growth conditions commonly used for cells in suspension or cells that are anchorage-independent.

The negative results of Example 2 confirm that TALL-104 cells do not behave as standard suspension cells and that standard growth condition for suspension cultures could not be applied as a routine. In summary, since TALL-104 cells were known as very peculiar cells, the skilled artisan would not have had any reason to apply common large scale culturing conditions for suspension cells. Accordingly, Gambacorti-Passerini et al. would not have been considered in view of WO 94/26284 unless the solution as stated in the present Claim 1 was known. To conclude otherwise would be an impermissible hindsight construction. Furthermore, the Applicant has already pointed out that, since Gambacorti-Passerini et al. actually discloses a method for LAK cells activation on a large scale, the combination of Gambacorti-Passerini et al. and WO 94/26284 is not possible without the benefit of a hindsight construction.

In conclusion, the Applicant submits that TALL-104 cells, to which claim 1 is limited, represents a clonal population that is extremely different from other TALL clonal isolates. Moreover, the LAK cells disclosed by Gambacorti-Passerini et al. were from a LAK cell population, derived from a melanoma patient, see p. 523, 2nd column, end of par., which is at variance with TALL cells) and NK lymphocytes. Thus, the skilled artisan would not have applied the Gambacorti Passerini et al. teachings to the present TALL-104 population on the basis of the number of differences highlighted in WO 94/26284.

The combination of Gambacorti-Passerini et al. with WO 94/26284 would not have been made by one of ordinary skill in the art in view of the disclosure reported in Gambacorti-Passerini et al. reference itself. As a matter of fact, Gambacorti-Passerini et al. show that the

culture method using the multi-chamber stacks is faster and more affordable for LAK cells on the basis of eliminating some of the steps required for patient's PBL activation in flasks as well as in the possibility to activate patient's PBL also in presence of moderate or high granulocyte contamination (see Gambacorti-Passerini et al., Discussion section on page 529, column 1, lines 4-7, wherein Gabacorti-Passerini et al. state: "This method does not require the use of Ficoll, clears contaminating erythrocytes completely, and can be used in the presence of moderate granulocyte contamination." The reference further states at page 529, column 2, lines 13-16: "The data we present here are important in this context in that they show: a) the possibility of eliminating some steps in the activation procedure; b) the feasibility and affordable cost of this method...").

As far as the elimination of steps is concerned, according to Gambacorti-Passerini et al., this is principally accomplished by the adoption of "ACK treatment" as an alternative to the expensive and lengthy Ficoll separation affected by a marked loss of PBL, (see Gambacorti-Passerini et al., page 525, column 2, lines 5-9: "Thus it is possible to collect and activate PBL from leukaphereses without performing Ficoll separation which is known to cause a marked loss of PBL number and which does not completely clear contaminating erythrocytes.")

Furthermore, Gambacorti-Passerini et al. show that the highest activation is obtained with the use of a Cell-factory in the case of high granulocyte contamination of PBL in comparison to the activation in flask even if preceded by Ficoll purification (see Gambacorti-Passerini et al., page 527-528: "Lymphocytes cultured in the CF without Ficoll purification, developed the highest activation, .i e. slightly higher than obtained in the small flasks after Ficoll."; see also Table 7 at page 528).

However, in contrast to LAK cells, TALL-104 cells are an established and conserved cell line which does not need Ficoll separation and does not have any problem of (granulocyte/erythrocyte) contamination. Accordingly, when pursuing the large scale amplification of TALL-104, a skilled artisan would not have not considered the Gambacorti-Passerini et al. reference because of the differences in LAK cells vs. TALL-104 cells. Moreover, the main advantage achieved and declared in Gambacorti-Passerini et al. is not at all relevant to TALL-1 04 cells.

Culture Parameters

The Examiner further stated at page 4 to page 7 that the remaining features of Claim 1, such as the addition into the multi-chamber stack an inoculum of at least 0.7 x 10^6 TALL-104 cells/ml in an initial volume from 1/10 to 1/6 of the multi-chamber stack final volume capacity and the cell number amplification by addition of a complete medium volume corresponding to the volume contained in the multi-chamber stack every 3-5 days are obvious over Gambacorti Passerini, in view of Tuyaerts and Schumpp, respectively, in combination with the Nunc Cell Factories instruction for use. The Examiner supported the rejection on the basis that Tuyaerts et al. discloses Cell-Factories for use in cytokine dependent cells, as TALL-104 are also cytokine dependent cells, without specifically indicating the features of addition into the multi-chamber stack an inoculum of at least 0.7x10^6 TALL-104 cells/ml in an initial volume from 1/10 to 1/6 of the multi-chamber stack final volume capacity and the cell number amplification by addition of a complete medium volume corresponding to the volume contained in the multi-chamber stack every 3-5 days in Tuyaerts et al.

First of all, Tuyaerts et al. describes dendritic cells, which are not even T-lymphocytes. Therefore the Examiner has appeared to base her conclusions on the basis of a further hindsight, because a combination with W094/26284 or Gambacorti-Passerini et al. would not have been made by a skilled person, especially since Tuyaerts et al. does not pertain to the relevant field of endeavor. TALL-104 cells are not only distinctly different from LAK cells as disclosed by Gambacorti-Passerini et al., they are also quite different with regard to the dendritic cells (DC) as disclosed by Tuyaerts et al.

The Examiner stated on page 5 that "Schumpp et al. teach that the amplification of cells in culture results in depletion of nutrients and the accumulation of toxic compounds" and that "it would have been obvious to optimize the method by increasing the volume of medium added, since the growth of cells in culture results in a loss of nutrients and an accumulation of toxic components." According to the Examiner, the feature of adding a complete medium volume corresponding to the volume contained in the multi-chamber stack every 3-5 days would be extracted from Schumpp, which relates the optimization of the conditions in suspension of the HL-60 cell line for high density production. The skilled artisan would not have combined WO 94/26284, which relates to a method for modifying TALL-1 04 cells, with Gambacarorti-

Passerini et al., which relates to LAK cell culture, or with the teachings of Tuyaerts et al., which relates to Dendritic Cells. Such a combination would not have made by the skilled artisan, who would have been aware of the different peculiarities of the cells, such that there would have been no expectation of success.

In the Office Action, on page 9, the Examiner noted that the total volume capacity of the Nunc Cell Factory™, used by the cited references is closer to the Applicant's calculation, based on the internal dimensions. In the Applicant's view these calculation are meaningless, in view of the fact that Claim 1 comprises a cell density to be achieved, and not a specific final volume. Moreover, the Applicants note that the Examiner's assumption that: "as much as 20% of the total height is lost due to the physical space occupied by the stacked chambers..." (see page 9) is just an arbitrary calculation which is not supported by any sound proof.

Alternatively, by applying cell density calculations, the teaching of Tuyaerts et al. becomes even more distant from the instant cell density values, as this parameter corresponds to not more than $5x10^6$ cells/chamber in Tuyaerts, while it is ($0.7x10^6$ TALL-104 cells/ml multiplied by 200 ml for each chamber) 1.5-2.5 x 10^7 cells/chamber.

Adherent Cells vs. Cells in Suspension

The Applicants point out that Tuyaerts et al. discloses DC amplification in a Cell Factory but, at variance with TALL-104 growing in suspension, selects for the amplification of an adherent cell population (see, in particular, page 138, 1st col.: "The non-adherent cells were removed via a cell-collection tubing.... The adherent cells were cultured in 160 ml.... On day 6, cells were harvested as immature DC...etc." The Examiner stated at page 8, fourth full paragraph that, while the precursor cells described by Tuyaerts et al. are initially adherent, the actual dendritic cells harvested from the culture are non-adherent cells. However, TALL-104 cells exhibit very different growth properties in comparison to dendritic cells. Among the numerous differences, TALL-104 cells are preferentially grown in suspension. Thus, the skilled artisan would have had no sound reason for applying the teachings of Tuyaerts et al. to the culture of TALL-104 cells. The fact that the volume added in Tuyaerts et al. roughly corresponds to the volume specified in Claim 1 does not support a case of *prima facie* in view of the fact that the skilled artisan would not have applied the teachings of Tuyaerts et al. for the reasons discussed above.

In view of the preceding remarks, the skilled artisan would not have combined the cited references to arrive at the presently claimed process. Thus the Claims are not obvious over the prior art of record.

Claim 14 was rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 94/26284 in view of Gambacorti-Passerini et al. (*supra*), Tuyaerts et al. (*supra*) and Schumpp, B. and Schlaeger, E.J. (*supra*) as applied above, in further view of U.S. Patent No. 6,491,678 (the '678 patent). The '678 patent teaches a freezing bag that can be sealed to create sample chanber that can be detached without thawing for testing the suitability of the frozen cells. However, in view of the remarks pertaining to WO 94/262843, Gambacorti-Passerini et al., Tuyaerts et al., Schumpp, B. and Schlaeger, E.J. and the product information of Nunc cell factories discussed above, Claim 14 is also not obvious.

In view of the foregoing remarks, one of ordinary skill in the art would have had no reason to combine the prior art to develop the presently claimed process. Hence, the claims are not *prima facie* obvious in light of the cited references of record. Accordingly, the Applicants respectfully request that the rejections under 35 U.S.C. § 103(a) be withdrawn.

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicant is not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicant reserves the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicant has made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

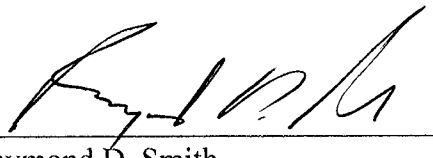
In view of Applicants' amendments to the Claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: August 20, 2009

By: 

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